Praziquantel Form B: preparation and characterization of the only ever-known anhydrous polymorph

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INTRODUCTION

Praziquantel (PZQ) is the antihelmintic drug of first choice against Schistosomiasis, an infectious diseases affecting at least 230 million people worldwide.[1] This drug is of low cost, highly efficient with little side effects, but it has, although, low solubility and extensive first-pass metabolism,[2] causing high dosages often difficult to swallow in children, the main target of this treatment. This study reports the only ever-known polymorph of PZQ, Form B, obtained by grinding in a vibrational mill. Considering different polymorphs of a drug, significant differences can be noticed, not only in the crystalline structure and arrangements at the solid state (which represent the necessary conditions of being) but more interestingly in the physical, chemical and biopharmaceutical properties.[3] Actually the known crystal structure of PZQ in the CSD are represented by the anhydrous commercial racemic compound (TELCEU), the enantiomeric hemihydrates (SIGBUG and SIGBUG01) some derivatives and cocrystal with dicarboxylic acids.[4] PZQ crystallization by slow evaporation in different solvents is reported to lead to the same raw drug.[5] In this study the crystalline structure of Form B was solved from the powder X-ray diffraction pattern (CSD dep. n. 1557658) then fully characterized both at the solid state and in its biopharmaceutical properties (i.e. saturation solubility and intrinsic dissolution rate) comparing to raw PZQ. Finally, the antihelmintic activity both *in vitro* and *in vivo* (in mice) was tested.

MATERIALS

Praziquantel Ph. Eur. Grade (Fatro S.pA., Bologna, Italy); HiPersolv Chromanorm Methanol, Ph. Eur. for HPLC Gradient Grade (VWR Chemicals BHD PROLABO®).

METHODS

Form B preparation

PZQ Form B was prepared via neat grinding of raw PZQ in a Retsch MM400 (Retsch GmbH) vibrational mill, using 2 screw-type zirconium oxide jars of 35 ml and 3 zirconium oxide balls (Ø=15 mm).

Form B characterization

The solid state of PZQ Form B was fully characterized by DSC, TGA, polarimetry, ATR-IR, FT-IR (KBr), Scanning Electron Microscopy (SEM), SS-NMR and powder X-ray diffraction using conventional and synchrotron sources. For the determination of the drug content a Shimadzu reversed-phase HPLC-UV was used, with a mobile phase of methanol:water (65:35 V/V). The water solubility was tested at 20 °C while and the intrinsic dissolution rate at 37 °C, using in both cases the same HPLC-UV system for drug quantification. Finally, the activity of Form B was evaluated both *in vitro* and *in vivo* (in mice) against *S. mansoni*.

RESULTS AND DISCUSSION

PZQ Form B is the only ever-known anhydrous polymorph of PZQ: its PXRD pattern was clearly different form raw PZQ since the characteristic peak at 4 of 2θ disappeared while two intense peak at 6.9 and 8.5 of 2θ were evidenced. The solution of the new phase from the powder X-ray diffraction pattern revealed the presence of one molecule in the asymmetric unit, in a monoclinic space group C2/c (see Figure 1). Conversely, raw PZQ has a triclinic unit cell (P-1) where 4 crystallographically independent molecules are arranged, of which 2 are disordered.[4] The crystal habit of Form B observed by SEM revealed an agglomeration of whiskers, different from the acicular shape of raw PZQ. The anhydrous nature of Form B was confirmed by DSC and TGA, with a single event at 112 °C corresponding to

the melting of the polymorph. A similar melting point and crystal space group was reported for the enantiomeric hemihydrate of PZQ, but the polarimetric analysis of Form B showed no enantiomeric excess. The total amount of impurity, analyzed by HPLC, resulted in less than 1%.

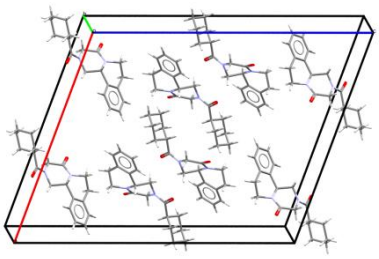

Figure 1. Praziquantel Form B unit cell (C2/c).

The SSNMR (^{13}C and ^{15}N) confirmed the novelty and crystallinity of Form B and also the presence of one independent molecule in the unit cell, since a single set of resonances in ^{13}C was evidenced. The ATR-IR spectra of Form B comparing to raw PZQ evidenced again the difference between the two phases, particularly in the carbonyl stretching range. The high resolution FT-IR permitted to highlight also a different crystal conformation of the two phases: in fact the C=O groups in raw PZQ are in the *syn* conformation, while in Form B the *anti* conformation gives origin to a closer double peak (1642-1632 cm^{-1}) than the one of PZQ (1653-1628 cm^{-1}). The biopharmaceutical properties of the new form were analyzed and compared to raw PZQ. The solubility in water of the new phase at 20 °C (after 48h under stirring in the dark) was double than raw PZQ with a value of 281.31 mg/L comparing to 140.30 mg/L. The intrinsic dissolution rate (37 °C) followed the same trend with a value of 0.062 ± 0.0042 mg*cm^{-2}/min for Form B and of 0.0312 ± 0.003 for raw PZQ (see Figure 2).

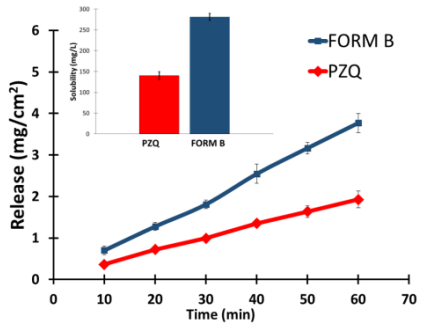

Figure 2. Water solubility and intrinsic dissolution rate of PZQ and Form B.

The *in vitro* and *in vivo* activity of Form B against *S. mansoni* was maintained and enhanced, comparing to raw PZQ, as showed in Table 1. Finally, the new phase showed to be physically stable (by PXRD and DSC) for more than one year.

Sample	In vitro IC50 (72h) ng/ml	In vivo (mice) % Schistsomule reduction after 49 days treatment
PZQ	165	96.8
Form B	135	100.0

Table 1. *In vitro* and *in vivo* activity of PZQ and Form B against S. Mansoni.

CONCLUSIONS

This work reports the only ever-known polymorph of PZQ (Form B), obtained through a mechanochemical process in a vibrational mill. This new phase, after being fully characterized at the solid state, was evaluated not only in its biopharmaceutical properties, but also in its activity both *in vitro* and *in vivo* against *S. mansoni*. Form B has a double solubility and intrinsic dissolution rate in water than raw PZQ, slightly enhancing the antischistosomal activity. These interesting properties of Form B, together with its stability for more than one year make this new form an interesting product for the design of new PZQ formulations.

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REFERENCES

1. Nogi, T.; Zhang, J.; Chan, J. D.; Marchant, J. S. A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel b subunits: subversion of flatworm regenerative polarity PLoS Negl. Trop. Dis., 3 (6), e464, 1-13 (2009).
2. Trastullo, R.; Dolci L.S.; Passerini, N.; Albertini, B. Development of flexible and dispersible oral formulations containing praziquantel for potential schistosomiasis treatment of pre-school age children Int. J. Pharm., 495 (1) 536-550 (2015).
3. Bernstein, J. Polymorphism in molecular crystals. Polymorphism in Molecular Crystals, Clarendon Press: Oxford, 2002.
4. Espinosa-Lara, J. C.; Guzman-Villanueva, D.; Arenas-García, J. I.; Herrera-Ruiz, D.; Rivera-Islas, J.; Román-Bravo, P.; Morales-Rojas, H.; Höpfl, H. Cocrystals of Active Pharmaceutical Ingredients Praziquantel in Combination with Oxalic, Malonic, Succinic, Maleic, Fumaric, Glutaric, Adipic, And Pimelic Acids. Cryst. Growth Des., 13, 169−185 (2013).
5. Toro, R.; Kaduk, J.; Delgado, M.; Díaz de Delgado, G. Structural characterization of praziquantel: a broad spectrum anthelmintic, in powder diffraction. Powder Diffr. 2014, ICDD Annual Spring Meetings, 29 (2), 206-207